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Effects of municipal waste water irrigation on availability of heavy metals and morpho-physiological characteristics of *Beta vulgaris* L.

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Abstract: *In the present study physiological, biochemical and growth characteristics of a leafy vegetable palak (Beta vulgaris L. var All green H1) grown in suburban areas irrigated by wastewater were compared with those irrigated by ground water. Continuous use of wastewater for irrigation led to the enrichment of micronutrients including heavy metals in the soil. Wastewater irrigation favorably affected the physiological, biochemical and growth characteristics of plants, but biomass and yield did not differ significantly between the sites. Uptake and translocation ratio of heavy metals were higher in plants grown at wastewater irrigated site. Mn showed maximum uptake followed by Zn, Cu, Pb, Ni Cr and Cd. Plants produced more secondary metabolites and antioxidants to tolerate against the negative impact of heavy metals at wastewater irrigated sites. Plants produced more metabolites to compensate the toxicity of metals in the area and thus did not enhance the yield and biomass potential. The study suggests that plants growing in wastewater irrigated area have potentially developed the defense strategy to combat against heavy metal toxicity.*

Key words: *Wastewater, Heavy metal uptake, Beta vulgaris, Antioxidant, Growth, Photosynthesis*
PDF of full length paper is available online

Introduction

Sewage and industrial wastewater is commonly used for irrigating agricultural fields in developing countries including India (Sharma *et al*., 2007; Pandey *et al.,* 2008; Nath *et al*., 2009; Nagajyothi *et al*., 2009). Continuous use of wastewater leads to the enrichment of soil with essential macro and micro-nutrients (Dass and Kaul, 1992; Kanan *et al*., 2005). Micro-nutrients are beneficial for the growth and metabolism of the plants at lower concentrations, but become toxic at excess than the requirement. Several micronutrients are heavy metals and known to produce undesirable effects on plants at higher concentrations (Kocak *et al*., 2005).

Accumulation of toxic heavy metals leads to stress conditions in the plant system by interfering with the metabolic activities and physiological functioning of the plants. Heavy metals are known to cause membrane damage, structural disorganization of organelles, impairment in the physiological functioning of the plants and ultimately growth retardation (Kimbrough *et al*., 1999; Chien and Kao, 2000; Long *et al*., 2003; Zhang *et al*., 2002). Heavy metals stimulate the formation of reactive oxygen species (ROS) such as superoxide radicals (O_2°), hydroxyl radicals (OH°) and hydrogen peroxide (H_2O_2) either by transferring electron involving metal cations or by inhibiting the metabolic reactions controlled by metals (Stohs and Bagchi, 1995; Verma and Dubey, 2001). In order to survive under the stress condition, plants have enzymatic and non enzymatic antioxidants to scavenge free radicals (RS°, H_2O_2) and reactive oxygen species (O^-, O_2°, OH°). Measurement of antioxidants as stress markers is an important aspect for assessing the stress responses in plant system (Gratao *et al*., 2005).

Perishable vegetables are often grown around urban areas, which are more prone to heavy metal contamination due to variety of urban and industrial activities including vehicular pollution. Continuous use of wastewater for irrigation leads to the accumulation of heavy metals in the vegetables (Singh and Kumar, 2006; Sharma *et al*., 2006, 2007; Gupta *et al*., 2009).

The present study was conducted to compare the physiological, biochemical, growth and yield responses of a perishable leafy vegetable palak (*Beta vulgaris* L. All green H1) grown in suburban areas of Varanasi having long term irrigation of wastewater and ground water from bore well. The study also quantified the impact of wastewater irrigation on uptake and translocation of heavy metals in the plants.

Materials and Methods

Study area: The experiment was conducted at the suburban areas of Varanasi (25°18´N latitude and 83°01´E longitude and 76 m above the sea level) located in eastern Gangetic plain of India. The experimental sites selected are Dinapur (northeast of the city center) and Lohta (west of the city center). The experiment was conducted during October to December 2004. During the study period the range of maximum and minimum temperature were 20.0-26.0°C and 8.30-12.4°C. There was no rainfall recorded during the study period. Relative humidity ranged from 81-89%. At Dinapur site, the irrigation of the agricultural field was done by water discharged from a Dinapur sewage treatment plant (DSTP) of 80 million liters day^{-1}

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(MLD) capacity installed in 1986. The treatment plants not only receive domestic sewage, but also effluents discharged from small scale fabric, plastic, battery industries, dyeing, metal plating, bicycle tires and heavy agricultural equipments located in the urban areas of Varanasi. Three sub sites were selected at Dinapur area (DW_1, DW_2 and DC). At site DW_1, both ground as well as treated sewage water is used for irrigation, while at DW_2 site only treated water is used for irrigating agricultural fields. Due to power failure some times untreated water is also discharged for irrigation. At Lohta (LW) site, irrigation was done with the untreated domestic sewage mixed with untreated wastewater from an industrial area and treated water of a large Diesel locomotive works (DLW), manufacturing diesel engines. At both DC and LC site ground water (bore well water) was used for irrigating the agriculture field.

Experimental set up and raising of plants: A plot of 6.5 × 4.5 m^2 size was prepared at each site. The plot was then divided into 6 subplots of 1.5 × 1.5 m^2 having margins of 0.50 m. Genetically uniform seeds of palak (*Beta vulgaris* L. var All green H1) procured from Indian Institute of Vegetable Research, Varanasi were sown in 5 rows at 2 cm depth in each sub plots. The water table of ground water source (bore well) was 76 m at both the sites. Uniform irrigation schedule was followed at all the sites to maintain similar moisture condition throughout the growth of plants.

Growth characteristics and biomass accumulation: For plant sampling, three replicates from each sub plot were collected randomly from both ground and wastewater irrigated sites. Root and shoot length and number of leaves were quantified. Leaf area was determined by using portable leaf area meter (Model LI 3100, LICOR, USA). For biomass estimation, plant samples were separately washed to remove the soil particles and separated into roots and shoots and oven dried at 80°C till constant weight was achieved. Plant parts were weighed separately and biomass was expressed as g $plant^{-1}$. Yield was calculated as fresh weight (g $plant^{-1}$) of edible portion of plant after harvesting at 40 days after germination (DAG).

Physiological and biochemical characteristics: Photosynthetic rate (Ps), and stomatal conductance (Cs) were measured on fully expanded leaves using portable photosynthetic system (LI-6200, LICOR, Inc., Lincoln, NE, USA) at ambient climatic conditions between 9 and 11 a.m. at photosynthetically active radiation ranging from 1000 to 1200 μ mol m^{-2} s^{-1} on three randomly selected plants from each plots at 40 DAG. The chlorophyll fluorescence measurements were also performed on the same leaf during 10-11 a.m. using portable plant efficiency analyzer (MK 29414, 174 Hansatech Instrument Ltd, U.K). Initial fluorescence (Fo), maximal fluorescence (Fm), variable fluorescence (Fv = Fm-Fo) and Fv/Fm ratio were measured.

Fresh leaves were sampled manually at 40 DAG for estimations of photosynthetic pigments, lipid peroxidation and different metabolites. Chlorophyll and carotenoid pigments were extracted in 80% acetone and estimated according to the methods of Maclachlan and Zalik (1963) and Duxbury and Yentsch (1956), respectively. Ascorbic acid, protein and proline contents were determined by the methods described by Keller and Schwager (1977), Lowry *et al*. (1951) and Bates *et al*. (1973) respectively. Total phenol content was measured using the method of Bray and Thorpe (1954). The methods of Britton and Mehley (1955) and Fahey *et al*. (1978) were used for analyzing peroxidase activity and thiol content, respectively. The lipid peroxidation was measured as melondialdehyde (MDA) concentration (Heath and Packer, 1968).

Heavy metal analyses of plant samples: Dried plant samples (1 g) were digested with 10 ml of ternary acid (HNO_3, H_2SO_4, and $HClO_4$ mixture in 5:1:1 ratio) at 80°C until a transparent solution was obtained (Allen *et al*., 1986). Water samples (50 ml) were digested in 10 ml of concentrated HNO_3 at 80°C until the solution became transparent (APHA, 2005). The concentrations of heavy metals in filtrate were determined by using atomic absorption spectrophotometer (Model 2380, Perkin 203 Elmer, Inc., Norwalk, CT, USA), To calibrate the instrument, blank and drift standards (Sisco Research Laboratories Pvt. Ltd., India) were run after five determinations to calibrate the instrument. The coefficients of variations of replicate analysis were determined for different determinations for precision of analysis and variations were found to be less than 10%.

Uptake and translocation ratio of heavy metals in the plant samples: Uptake and translocation of heavy metals were calculated at the time of final harvesting by the following formula (Singh and Agrawal, 2007):

$$\text{Uptake of heavy metal } (\mu g \text{ plant}^{-1} \text{ d}^{-1}) = \frac{M_2 W_2 - M_1 W_1}{T_2 - T_1}$$

Where M_1 and M_2 are metal concentrations ($\mu g\ g^{-1}$) in plant tissue and W_1 and W_2 is plant biomass (g) at initial T_1 and final T_2 samplings.

Translocation ratio was calculated by dividing the heavy metal concentrations in shoot by root.

Statistical analyses: Statistical analysis was done using SPSS programme (version 11). Significance of differences in measured parameters between ground and wastewater irrigated sites were assessed by conducting one way analysis of variance (ANOVA) followed by Duncan's multiple range test at 5% level. Correlations were calculated between heavy metal concentration and biochemical parameters of plants.

Results and Discussion

The irrigation water had a lower pH for wastewater than ground water at both sites. Wastewater showed higher concentration of heavy metals as compared to ground water (Table 1). The trend of heavy metal concentrations in wastewater was ZN>Mn>Cu>Cr>Pb>Cd>Ni at Dinapur, whereas at Lohta the concentration was maximum for Cr followed by Mn, Zn, Pb, Ni, Cu and than Cd (Table 1). Soil pH was lower at wastewater irrigated sites than respected ground water irrigated sites. Wastewater irrigated soil samples showed higher concentration of heavy metals as compared to ground water irrigated ones (Table 1).

Uptake of heavy metals were higher in the plants grown at wastewater irrigated sites as compared to the ground water irrigated ones (Fig. 1). The higher concentrations of heavy metals at wastewater sites are due to the effluents discharged from various heavy metal based industries into sewage water. Elevated levels of heavy metals in soil have been shown to increase the metal uptake tendency in the plants (Xians, 1989; Fytianos *et al*., 2001). The uptake and mobility of heavy metals from soil to plants depends on their bioavailability, which is affected by the total concentration of heavy metals, clay, organic carbon content and redox potential of the soil (Narwal and Singh, 1998). As compared to the sites at Dinapur (DW_1 and DW_2), uptake of all heavy metals except Cr was lower at LW site. This difference may be due to higher pH of soil at Lohta site. Soil pH is known to be negatively correlated with solubility of metals and their uptake from soil (Hough *et al*., 2003).

Translocation ratio was also higher at wastewater irrigation sites, and it was highest for Mn followed by Cd, Ni, Pb, Zn, Cr and then Cu (Fig. 1). Translocation ratio was more than one at all the sites, suggesting more concentrations of heavy metals in shoot than root portion. Mostly the heavy metals tend to remain in root portion of the horticultural crops (Paivoke, 2003), but an opposite trend was observed in the present study. This may be ascribed to very fast growth rate of the test leafy vegetable in a short period of time and higher biomass of above ground than below ground portion.

Plants grown at wastewater irrigated sites showed higher MDA concentration, an indicator of lipid peroxidation as compared to those grown at ground water irrigated sites (Table 3). Heavy metals are known to induce generation of ROS and free radicals, which can cause peroxidation of lipid membrane leading to increased permeability and oxidative stress to the plants (Nada *et al*., 2007). Enhancement in MDA concentrations in leaves of *Bruguiera gymnorrhiza* and *Kandelia candel* were also reported due to multiple heavy metals, such as Pb^{2+}, Cd^{2+} and Hg^{2+} stress (Zhang *et al*., 2007). Lipid peroxidation showed significant positive correlations with all the metal concentrations (Table 4).

Plants grown at wastewater irrigated sites showed higher levels of antioxidant metabolites to nullify the negative effects of heavy metals. Proline content was higher in plants grown at wastewater irrigated sites than ground water irrigated ones (Table 3). Proline content was found to be positively correlated with the concentration of Cr (r^2 = 0.506) (Table 4). Increased levels of heavy metals are known to affect permeability of membranes, which may lead to a water stress like condition inducing the production of proline (Basak *et al*., 2001). Other possible positive roles of proline include stabilization of proteins (Anjum *et al*., 2000), scavenging of hydroxyl radicals (Smirnoff and Cumbes, 1989) and regulation of NAD/NADH ratio (Alia and Saradhi, 1993).

Table - 1: Heavy metal concentrations (µg ml^{-1}) in ground (nd) and wastewater

Heavy metals	Dinapur waste water	Lohta waste water	Indian standard for heavy metals*
Cd	0.045 ± 0.001	0.04 ± 0.002	0.01
Cu	0.055 ± 0.002	0.035 ± 0.001	0.05
Pb	0.033 ± 0.001	0.065 ± 0.003	0.1
Zn	0.147± 0.002	0.097 ± 0.003	5
Mn	0.070 ± 0.001	0.12 ± 0.01	0.10
Ni	0.03 ± 0.001	0.045 ± 0.001	-
Cr	0.04 ±0.002	0.146 ± 0.02	0.05

*(Awashthi, 2000); nd : Not detectable, Mean of 3 replicates ±1S.E.

Phenol and thiol contents were also higher in plants of wastewater irrigated sites (Table 3). Increase in phenolic components was also reported in *Albizia lebbek* due to heavy metals such as Ni, Cr and Hg (Tripathi and Tripathi, 1999). Thiol content was positively correlated with concentrations of all the heavy metals (Table 4). Thiols do not represent a single compound, but are sulphur containing polypeptides, which are known as phytochelatins. Cysteine, a -SH containing amino acid is a key constituent of phytochelatins and plays an important role in metal detoxification. Phytochelatins are involved in the detoxification of heavy metals (Kneer and Zenk, 1992) by immobilizing the metal ions and facilitating their further transport to the vacuolar portion (Ortiz *et al*., 1995).

Ascorbic acid was higher in plants of wastewater irrigated sites (Table 3). Ascorbic acid, a natural antioxidant scavenges free radicals generated by heavy metals (Halliwell and Gutteridge, 1993). Ascorbic acid content in plant and metal concentration was positively and significantly correlated with Cd, Pb, Zn and Ni concentrations (Table 4). Sinha *et al.* (2007) have also reported higher production of ascorbic acid in fenugreek plants grown in soil amended with tannery sludge to nullify the adverse effects of heavy metals. *Colocasia esculentum* and *Raphanus sativus* grown in wastewater irrigated area of Durgapur, West Bengal showed higher production of ascorbic acid under wastewater irrigation (Gupta *et al*., 2009).

Total chlorophyll and carotenoid were higher in plants of wastewater irrigated sites (Fig. 2). Carotenoid is photosynthetic pigment, also functions as non enzymatic antioxidant protecting plants from oxidative stress by changing the physical properties of photosynthetic membranes with involvement of xanthophyll cycle (Gruszecki and Strzatka, 1991). An increase in carotenoid content is suggested a defense strategy of the plants to combat metal stress (Sinha *et al*., 2007).

Antioxidative enzyme, peroxidase also showed increment in its activity in plants grown at wastewater irrigated sites as compared to those at ground water irrigated ones (Table 3). Peroxidases play a significant role in defense against oxidative stress and are suggested to be indicators of metal toxicity (Radotic *et al*., 2000). Increase in peroxidase activity under heavy metal stress has also been reported in palak (*Beta vulgaris* var All green) grown at

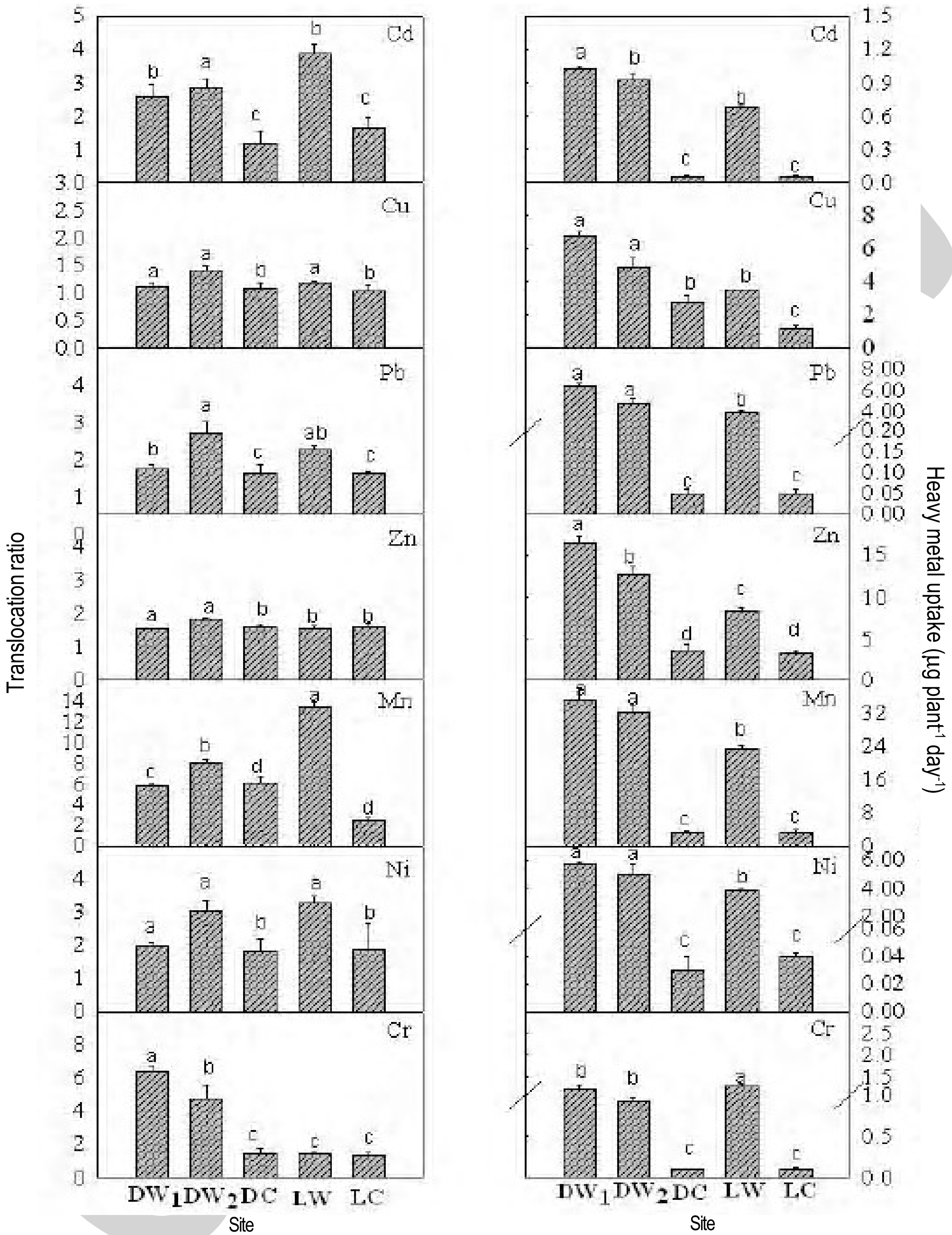

Fig. 1: Translocation ratio (TR) and heavy metal uptake rate of *Beta vulgaris* palak plants at ground and wastewater irrigated sites, DW_1 = Treated waste water+ground water, DW_2 = only treated wastewater, DC = Ground water at Dinapur, LW = Untreated waste water at Lohta, LC = Ground water at Lohta, Means of 3 replicates ±1SE. Bars with different letters in each group show significant difference at $p \leq 0.05$

different application rates of sewage sludge (Singh and Agrawal, 2007). Peroxidase activity showed positive and significant relationship with all the metal concentrations in plants (Table 4). Positive relationship suggests that with increase in the heavy metal concentrations, there were increase in the oxidative modifications to cellular components of the plants (Moller *et al*., 2007).

The photosynthetic rate and stomatal conductance were higher in plants at wastewater irrigated sites as compared to ground water irrigated ones (Fig. 3). The positive response of physiological characteristics of the plants at wastewater irrigated site suggest that the concentrations of toxic heavy metals may not be up to the extent causing adverse effects on photosynthetic apparatus. The higher level of antioxidants in plants at wastewater irrigated site may have reduced the negative effects of ROS on photosynthesis. The significant increases in photosynthetic as well as growth rate of plants grown at wastewater irrigated sites compared to ground water irrigated ones led to higher uptake and translocation of heavy metals in plants.

Fv/Fm ratio represents the efficiency of photosystem II. There were no significant variations in Fv/Fm ratio of plants under wastewater and ground water irrigation sites (Fig. 3). In the present study it varied from 0.77 to 0.85, which clearly showed the unstressed

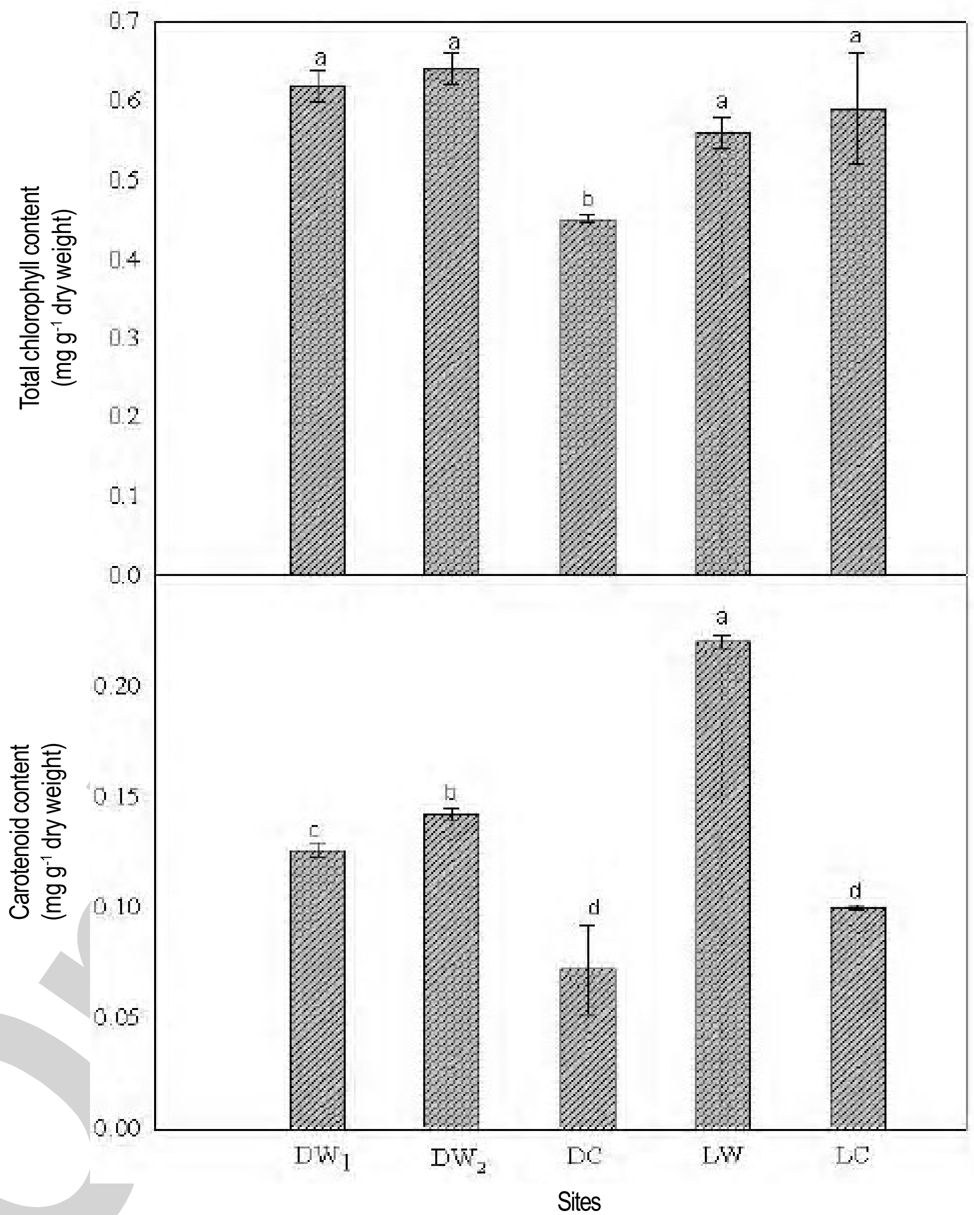

Fig. 2: Total chlorophyll and carotenoid contents in *Beta vulgaris* plants grown at ground and wastewater irrigated sites, DW_1 = Treated waste water+ground water, DW_2 = only treated wastewater, DC = Ground water at Dinapur, LW = Untreated waste water at Lohta, LC = Ground water at Lohta, Means of 3 replicates ±1SE. Bars with different letters in each group show significant difference at $p \leq 0.05$

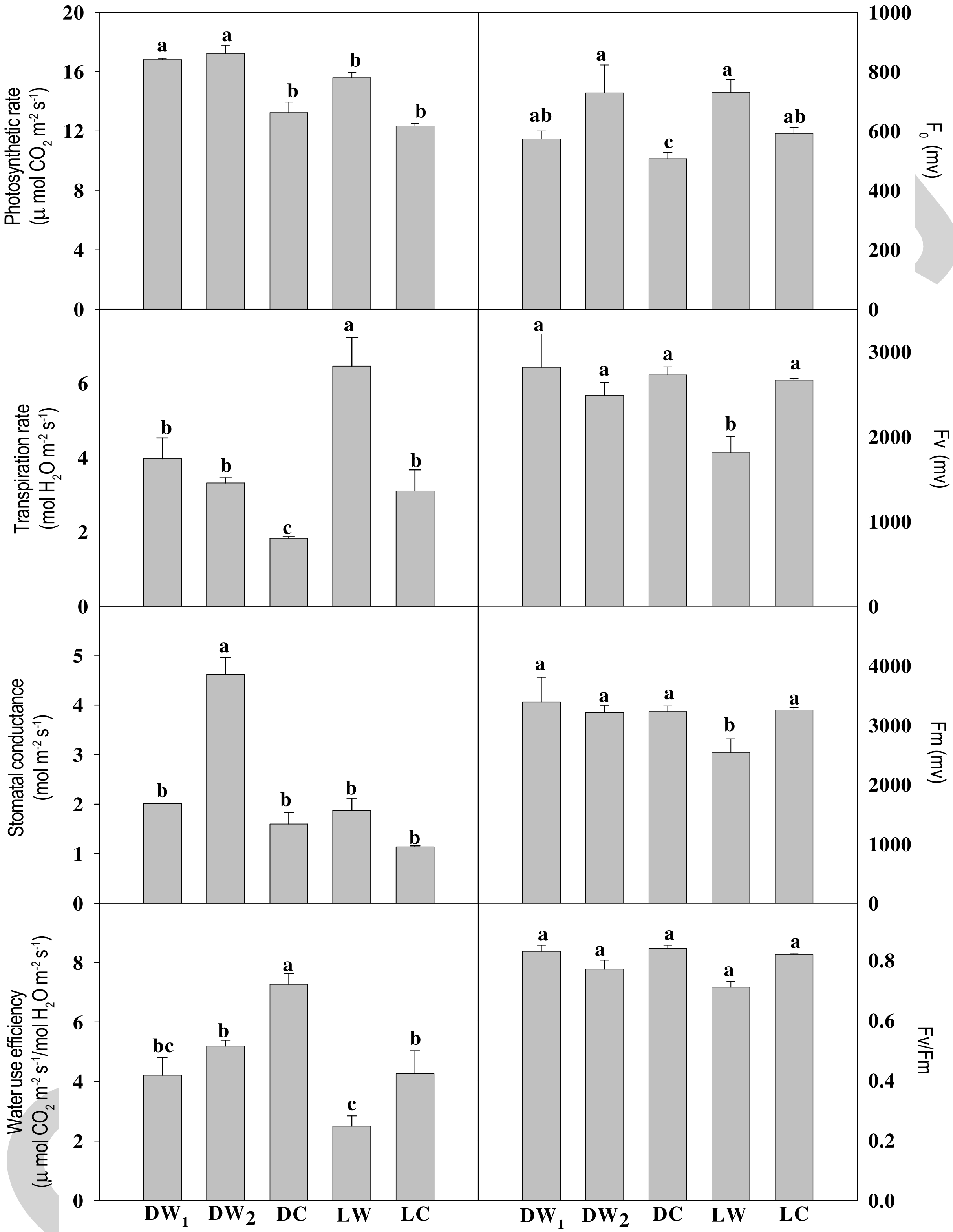

Fig. 3: Physiological characteristics of *Beta vulgaris* plants grown at ground and wastewater irrigated sites, DW_1 = Treated waste water+ground water, DW_2 = only treated wastewater, DC = Ground water at Dinapur, LW = Untreated waste water at Lohta, LC = Ground water at Lohta, Means of 3 replicates ±1SE. Bars with different letters in each group show significant difference at $p \leq 0.05$

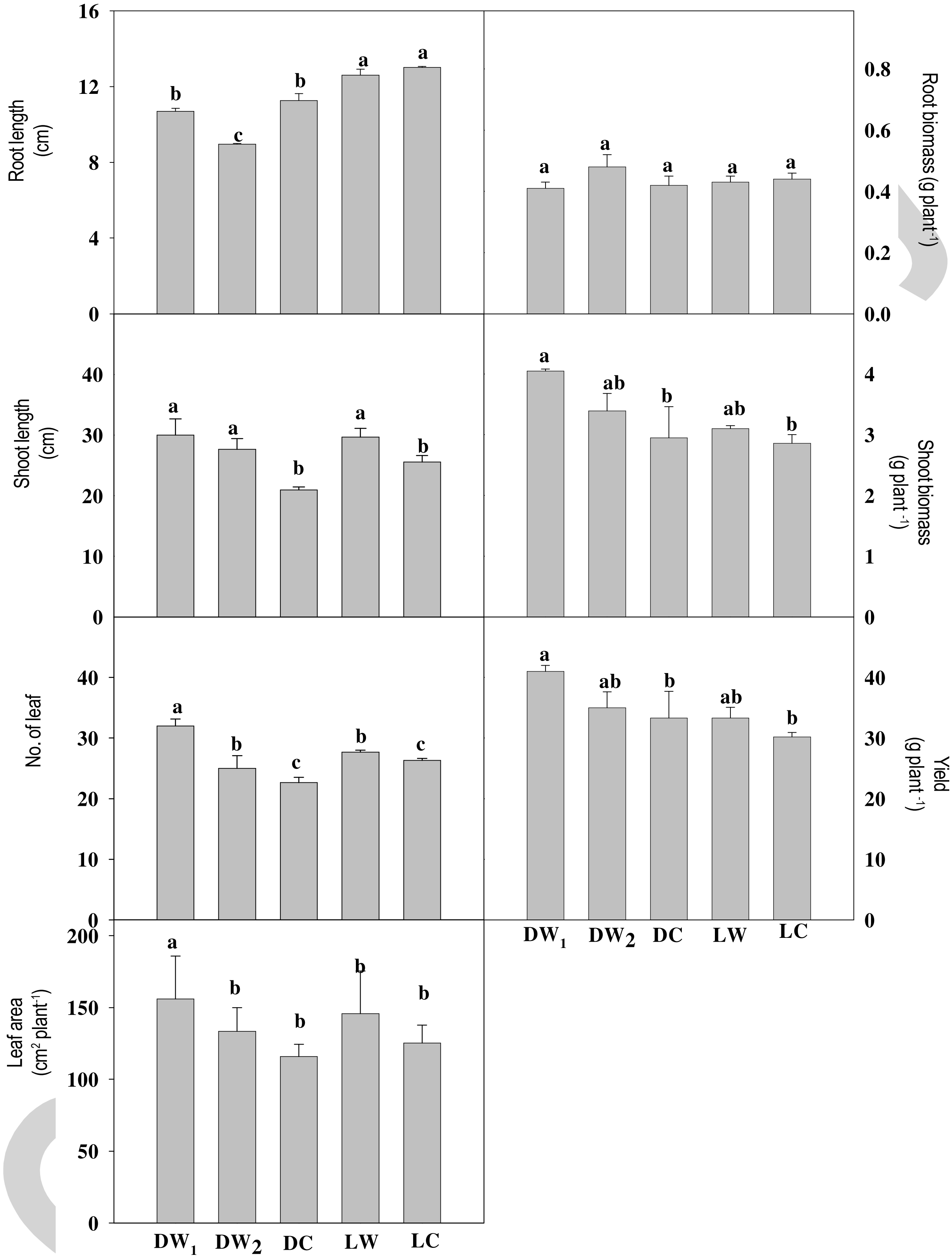

Fig. 4: Morphological characteristics of *Beta vulgaris* plants grown at ground and wastewater irrigated sites, DW_1 = Treated waste water+ground water, DW_2 = only treated wastewater, DC = Ground water at Dinapur, LW = Untreated waste water at Lohta, LC = Ground water at Lohta, Means of 3 replicates ±1SE. Bars with different letters in each group show significant difference at $p \leq 0.05$

Table - 2: Heavy metal concentrations (µg g^{-1}) in ground and wastewater irrigated soil of experimental sites

Heavy metals	DW_1	DW_2	DC	LW	LC	Indian standard for heavy metals*
Cd	5.60 ± 0.07	5.52 ± 0.09	1.75 ± 0.12	6.60 ± 0.12	1.54 ± 0.02	3-6
Cu	16.43 ± 0.17	19.90 ± 0.50	7.15 ± 0.33	21.40 ± 0.40	6.65 ± 0.10	135-270
Pb	25.50 ± 0.98	26.27 ± 1.20	8.30 ± 0.05	21.75 ± 0.45	8.35 ± 0.05	250-500
Zn	28.40 ± 0.55	27.55 ± 0.60	11.12 ± 0.55	43.35 ± 0.25	14.20 ± 0.25	300-600
Mn	466.20 ± 12.35	625.05 ± 14.20	116.80 ± 2.40	750.55 ± 18.12	112.50 ± 6.55	- - -
Ni	16.95 ± 0.30	19.30 ± 0.25	8.80 ± 0.03	15.30 ± 0.75	8.50 ± 0.09	75-150
Cr	15.9 ± 0.15	17.83 ± 0.25	8.65 ± 0.05	40.12 ± 1.25	8.92 ± 0.01	- - -

DW_1 = Treated wastewater + ground water, DW_2 = Only treated wastewater, DC = Ground water, LW = Untreated wastewater, LC = Ground water, Mean of 3 replicates ± 1SE. Different letters in each row showed significant difference at p≤0.05

Table - 3: Contents of selected metabolites, lipid peroxidation and peroxidase activity in *Beta vulgaris* plant grown at different experimental sites

Parameters	DW_1	DW_2	DC	LW	LC
Peroxidase activity (µm purpurogallin min^{-1} g^{-1} fresh leaf)	20.58^{a}±1.65	23.38^{a}± 0.83	8.07^{c}± 0.33	13.58^{b}± 0.87	4.12^{d}± 0.82
Proline content (mg g^{-1} fresh leaf)	0.98^{b}± 0.01	1.19^{a}± 0.05	0.94^{b}± 0.09	1.46^{a}± 0.14	1.27^{b}± 0.03
Phenol content (mg g^{-1} fresh leaf)	2.51^{a}± 0.04	2.18^{b}± 0.08	2.01^{b}± 0.04	1.06^{c}± 0.19	0.88^{c}± 0.04
Ascorbic acid content (mg g^{-1} fresh leaf)	0.63^{a}± 0.03	0.62^{a}± 0.03	0.55^{b}± 0.09	0.54^{b}± 0.06	0.39 ^{c}± 0.02
Thiol content (µ mol g^{-1} fresh leaf)	2.34^{d}± 0.05	8.89^{a}± 0.16	6.73^{b}± 0.06	6.17^{b}± 0.49	4.18^{c}± 0.54
Lipid peroxidation (n mol ml^{-1} fresh leaf)	0.77^{a}± 0.11	0.77^{a}± 0.18	0.30^{b}± 0.04	0.71^{a}± 0.02	0.26^{b}± 0.09
Protein content (mg g^{-1})	13.69^{a}± 1.54	13.22^{a}± 0.72	13.39^{a}± 1.99	14.50^{a}± 0.15	13.58^{a}± 1.63

Different letters in each row showed significant difference (p ≤ 0.05), DW_1 = Treated wastewater + ground water, DW_2 = Only treated wastewater, DC = Ground water, LW = Untreated wastewater, LC = Ground water at Dinapur Mean of 3 replicates ± 1SE. Different letters in each row showed significant difference at p≤0.05

Table - 4: The values of correlation coefficient between selected biochemical parameters of *Beta vulgaris* plants and heavy metal concentrations

Metals	Peroxidase activity	Lipid peroxidation	Phenol	Protein	Proline	Thiol	Ascorbic acid
Cd	0.803**	0.778**	0.234^{NS}	0.207^{NS}	0.437^{NS}	0.854**	0.521*
Cu	0.834*	0.790**	0.253^{NS}	0.264^{NS}	0.461^{NS}	0.926**	0.473^{NS}
Pb.	0.854**	0.819**	0.307^{NS}	0.295^{NS}	0.399^{NS}	0.868**	0.572*
Zn	0.924**	0.693**	0.617*	0.606*	0.015^{NS}	0.770**	0.716**
Mn	0.825**	0.787**	0.162^{NS}	0.215^{NS}	0.499^{NS}	0.845**	0.492^{NS}
Ni	0.769**	0.786**	0.225^{NS}	0.243^{NS}	0.473^{NS}	0.870**	0.541*
Cr	0.739**	0.763**	0.147^{NS}	0.166^{NS}	0.506*	0.786**	0.492^{NS}

Level of significance ** = p ≤ 0.001, * = p ≤ 0.01, NS = Not significant

condition for the photosynthetic apparatus of the plants (Demming and Bjorkman, 1987). This may be because the heavy metals may have been accumulated in portions of cells, not involved in the metabolic activities of the plants. Compartmentalization of heavy metals in cell organelles not responsible for important activities is known to reduce the negative impact on the growth and other cellular metabolic activities of plants (Vogeli-Lange and Wagner, 1990). Harmens *et al*. (1994) have reported that molecules in the cytoplasm like phytochelatins form complexes with metals and facilitate their transport over the tonoplast into the vacuole and do not cause any negative impact on the plants.

Shoot length and number of leaves of plants were significantly higher at wastewater irrigated sites as compared to the respective ground water irrigated ones (Fig. 4). Paliwal *et al*. (1998) have also found significant increase in shoot length of *H. binata* with the treatment of increased percentages of sewage water. Wheat plants irrigated by treated and untreated textile effluents showed increment in shoot length as compared to the plants irrigated by distilled water (Kaushik *et al*., 2005). Root length decreased in plants at wastewater irrigated sites as compared to the ground water irrigated ones (Fig. 4). The reductions in the root length may be due to increase in the nutrient concentrations under wastewater irrigation as compared to the ground water irrigated ones. Leaf area of plant was highest at DW_1 site, which clearly suggests that intermittent use of ground and wastewater has reduced the heavy metal accumulation in plant tissue leading to favorable growth compared to sites receiving continuous wastewater (Fig. 4).

There were insignificant differences in the yield and biomass accumulation of plants grown at ground and wastewater irrigated sites (Fig. 4). Heavy metals in soil may interfere with the nutrient uptake (Derome and Lindroos, 1998). Higher bioavailability of heavy metals in wastewater irrigated sites may have reduced the nutrient availability to plants that may be the cause for not showing significant increments in biomass of these plants as compared to the plants grown at ground water irrigated sites. The favorable physiological and growth responses are not translated into increments in the biomass accumulation and yield of plants, as the photosynthates are utilized in the formation of secondary metabolites to ameliorate the negative influence of heavy metals.

The antioxidative capacity of plants increased at wastewater irrigated sites to induced tolerance against heavy metals leading to positive effects on growth and photosynthesis. The favorable morpho-physiological responses are not translated into significant increase in the biomass accumulation and yield of the plants, as the primary metabolites are utilized in the formation of antioxidants and secondary metabolites to counteract the negative influence of heavy metals. This study thus suggests that higher avaibility of heavy metals restricts the usefulness of waste water for irrigation.

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